polarity-dependent process occurring during zebrafish gastrulation is directional cell division aligned along the anterior–posterior axis, which contributes in part to the elongation of the body axis [18].

William Smith's group [19] has previously reported that the *chobi* mutant *Ciona* embryos show a phenotype reminiscent of *aimless/prickle* mutant embryos. Further identification of *Ciona* mutants that exhibit a shorter tail phenotype with properly differentiated notochord could uncover novel members of the planar cell polarity pathway, as has been done for gastrulation defects in zebrafish and for neural tube defects in mice. Considering that the extent of genome redundancy in ascidians is similar to that in *Drosophila* with respect to planar cell polarity genes [20], this approach might be more successful in these species than in other vertebrates.

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Circadian Biology: Fibroblast Clocks Keep Ticking

Real-time cellular imaging of gene expression has revealed that fibroblasts contain a robust, self-sustained and cell-autonomous circadian oscillator, with a range of properties that both overlap and contrast with those of the neural clock of the suprachiasmatic nuclei.

Michael H. Hastings

Circadian clocks enable organisms to define biological day and night, synchronising daily rhythms of metabolism and behaviour to the demands and opportunities of the world [1]. Hence, clocks confer selective advantage and are hard-wired into our make-up. Our most obvious circadian rhythm is that of sleep and wakefulness, but they range from mucosal cell division through hormonal profiles to susceptibility to cardiac arrest. The circadian mantra used to be easy; "*there is but one true clock and it sits in the brain*". By using real-time cellular imaging, two papers [2,3] have revealed a new truth:, "*clocks are all over the body and all are equal, but some are more equal than others*".

After early skepticism that circadian rhythms are artefactual, the field was boosted in the 1980s with the identification of the hypothalamic suprachiasmatic nuclei (SCN) as the body clock controlling the sleep/wake and endocrine cycles [4]. Further respectability came in the nineties with the discovery of genes encoding the SCN clockwork [5]. Driven by complexes of the transcription factors CLOCK and BMAL, the 'clock genes' *Period* and *Cryptochrome* encode transcriptional inhibitors that oppose CLOCK/BMAL activity, thereby closing a negative feedback loop that oscillates with an approximately daily period. Electrophysiological recordings of dispersed cultures revealed that the clockwork is cell-autonomous, its activity within single SCN neurons indicating that there must

be something special about them [6]. This 'specialness' was not, however, reflected in their clock genes. Many other tissues also express these genes, with circadian profiles matching those of the SCN, albeit with tissue-specific phases [1]. Moreover, serum shock can initiate circadian gene expression in fibroblast cultures [7].

The idea of SCN specialness was knocked further with development of transgenic rodents in which the *Period1* promoter drives a luciferase reporter. Sure enough, as the circadian loop progresses through its transcriptional programme, alternately activating and suppressing *Period1*, transgenic SCN slices express beautiful circadian cycles of bioluminescence. The problem is that other tissues, including liver and lung, do the same [8]. With circadian clockwork being intrinsic to many body parts, sustainability was instead mooted as the critical difference between SCN, which 'tick' away for months, and peripheral tissue and fibroblast cultures, which quickly dampen. Even this criterion was shaky. A knock-in mouse, which had endogenous *PERIOD2* fused to luciferase, gave a stronger report of the clockwork and provided tissues that oscillated in culture for weeks [9].

The final test of cellular sustainability has now been made. Since the discovery by Ueli Schibler's group of circadian gene expression in fibroblasts, the damping was thought to reflect either attenuation of the individual cellular clocks, or their desynchronisation across the culture. To address this [2], they engineered a novel reporter gene with yellow fluorescent protein tagged with a nuclear localisation sequence and rendered unstable with a proteosomal degradation motif. Expression is controlled by sequences of the *Rev-Erbα* gene, the endogenous product of which is highly circadian and forms a link between the negative and positive elements of the clockwork [10]. In transfected NIH3T3 fibroblasts, this designer gene reports circadian time beautifully, nuclear

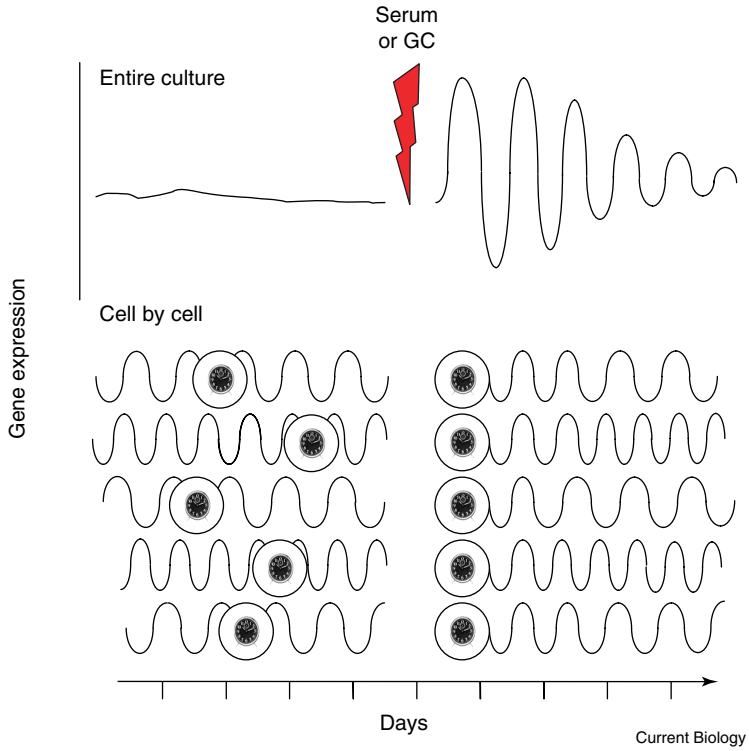

Figure 1. Intrinsic circadian clocks of peripheral cells.

Bioluminescent reporters of clock gene expression in long-term fibroblast cultures show little evidence of rhythmic activity, but cellular imaging reveals that under the surface individual cells within the culture have extremely robust and precise circadian clocks. These asynchronous clocks can be brought rapidly into tune by an acute serum shock or exposure to glucocorticoid (GC), leading to coherent circadian gene expression across the culture. Circadian coordination of metabolism *in vivo* probably relies on maintained synchronisation, by systemic signals, of otherwise weakly coupled, tissue-based cellular oscillators.

fluorescence waxing and waning on a daily basis. In cultures shocked with serum, the individual cellular clocks are tightly phased, but, importantly, in unperturbed cultures the cellular clocks are not dead. Rather, each nucleus glows and dims across a day, but the cells are out of tune with their neighbours. The conclusions, supported by further modelling data, are that cellular clocks are robust, that damping is a desynchronisation phenomenon, and that serum shocks do not initiate the cellular clocks, they simply re-synchronise them.

These conclusions are vindicated by further experimental data from Dave Welsh and colleagues [3]: Welsh first revealed with Steve Reppert the cell autonomous SCN clockwork [6]. By swapping a multi-electrode array for a highly sensitive CCD camera, Welsh *et al*. [3] recorded bioluminescence from individual cells in culture: Rat-1 fibroblasts transfected with *Bmal1::luciferase*, and primary embryonic fibroblasts from the *PERIOD2::LUC* mouse. The results are definitive; 10 days after serum shock circadian gene expression across the culture disappears, but when analysed in individual cells the clockwork cycles as strongly as ever. All that changes with time is that the cells drift apart, keeping to their individual circadian beats. So a humble fibroblast has a clock that can keep going for at least 15 days and probably much longer. Also a shot of serum can instantaneously reset the clock to a new phase, shifting some cells forwards or backwards by up to 12 hours: no jet-lag here!

It seems, therefore, that the specialness of SCN clocks is their ability to communicate. When cultured as an intact slice or dispersed cells at high density,

SCN neurons synchronise together via synaptic and possibly extra-synaptic communication [11,12]. Fibroblasts simply go their own way: both Nagoshi *et al*. [2] and Welsh *et al*. [3] show that the clockwork of any particular fibroblast is not influenced by its neighbours. In the real animal, some cellular communication may possibly occur, but more likely SCN-dependent cues such as glucocorticoid rhythms are the principal means of keeping the body's many clocks in overall tune.

Indeed, weak coupling and ability to make large phase-jumps may be adaptive features of peripheral clocks, whose primary function is to anticipate and respond rapidly to feeding. The hepatic clockwork controls many metabolic and detoxification genes [13,14] and is entrained by both SCN-dependent cues, such as glucocorticoid hormones, and feeding schedules [15,16]. In a harsh world, meal times may vary widely from day to day. The daily gene expression programme that allows the viscera to deal with the consequences of unpredictable digestion may need to react rapidly and so the ability to leap forward or backward will be advantageous. Tight coupling between cells would restrict this flexibility. Conversely, dawn and dusk entrain the SCN and vary little day to day. Small daily adjustments by the SCN are therefore sufficient to maintain synchrony with solar time and are consistent with tight cellular coupling. Indeed, SCN neurons send out multiple and cell-specific connections to numerous targets [17] and so tight coupling will be an asset, ensuring the time signal to each target is identical, thereby maintaining internal synchrony.

Does bodily synchrony have wider relevance? Proliferating tissues operate within two temporal domains; the circadian and the cell division cycles. Time-lapse imaging by Nagoshi *et al*. [2] shows that circadian time is passed from mother to daughter cells at division. Additionally, they show that cytokinesis, the separation of daughters, is restricted to one of three circadian times. Put another way, the rest of the circadian cycle is a 'no-go' area for cell division. Expression of many cell-division factors is circadian; here we see a likely consequence of that regulation. Moreover, disturbance of circadian coordination by genetic, environmental or surgical means promotes tumour progression in mice, and epidemiology indicates a link between shift-work and incidence of human cancers [1,18,19]. Not only have these single-cell imaging studies highlighted how fundamental circadian clocks are to cells, they also provide a platform to analyse how temporal domains of circadian and cell division cycle interact.

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